2098-Pos Board B84
A Hydrodynamic Analysis of the Human Cullin 5 - hIV-1 Vif Ubiquitin Ligase Complex
Stephen Techtmann, Rodolfo Ghirlando, Ernest Maynard.
The HIV accessory protein virion infectivity factor (Vif) is essential for viral replication in CD4+ cells. In the absence of Vif, HIV is efficiently restricted by the cellular cytidine deaminase, APOBEC3G (A3G). Vif recruits a cullin 5 (Cul5)-based ubiquitin ligase and Elongin B/C, which target A3G for proteasomal destruction. Many cullin-based ubiquitin ligases have been shown to oligomerize via their substrate binding motifs. It has also been reported that Vif oligomerizes, but the nature of these oligomers is unknown. We have employed biochemical and biophysical techniques to investigate the oligomeric properties of Vif and Cul5 alone and in complex.
Using analytical ultracentrifugation (AUC) we have characterized Vif, Cul5, and Elongin B/C and their complexes. This work will allow for an examination of how the oligomeric state of the free proteins effects the Vif-Cul5 interaction. From these studies we determined that Cul5 dimerizes with a Kd of 70 μM. Our initial studies with Vif (101-153) show little oligomerization. AUC analysis of the Elongin B/C shows that the predominant species is the heterodimer and some higher order oligomerization is observed. We have analyzed the Vif-Cul5-Elongin B/C complex and have seen that the stoichiometry of the complex is 1:1:1:1. Therefore it appears that the oligomeric forms of the individual proteins are not maintained in the complex. It is possible that the sites of self-association occur at the interfaces between the binding partners. To further characterize the Vif-Cul5-Elongin B/C interaction, isothermal titration calorimetry (ITC) was used to define the thermodynamic properties governing association. A thorough understanding of the mechanism of the Vif-Cul5-Elongin B/C complex will shed light on the possible role of oligomerization in both ubiquitin ligase function as well as the function of HIV-1 Vif.

2099-Pos Board B85
Expression and Purification of the BK Channel Alpha-Beta1 Complex
Yixuan Zhang, Yangyang Yan, Youshan Yang, Fred Sigworth.
Large conductance Ca2+ activated potassium channels (BK) are composed of pore-forming alpha-subunits and can be associated with modulatory beta-subunits. The beta1 subunit is mainly expressed in smooth muscle, and is found to increase the Ca2+ sensitivity of BK channels and slow the deactivation process. We have generated HEK293 cell lines stably expressing the hSlo alpha subunit and beta1 subunit genes. The hSlo construct carries N-terminal Flag and His tags for purification, while the mouse beta1 is fused with GFP at the C-terminus. Current recordings from inside-out patches showed a shift in the voltage sensitivity of activation consistent with co-assembly of the subunits. The alpha-beta1 complex was bound in the presence of decylmaltoside to Flag antibody affinity beads, and eluted with Flag peptide. Co-expression was verified by protein gels and western blots, and quantitative measurement of GFP fluorescence allowed the stoichiometry of the complex to be estimated.

2100-Pos Board B86
Assembly and Function of Pore-Forming Toxin Aerolysin from Aeromonas Hydrophila
Matteo Dal Peraro, Matteo Degiacomi, Ioan Iacovache, Gisou van der Goot.
One of the most ancient forms of attack between cells or organisms has been the production of proteins or peptides that affect the permeability of the target cell membrane. This class of weapons includes the largest family of bacterial toxins, the pore-forming toxins (PFTs). PFTs are bistable structures that can exist in a soluble and a transmembrane state. It is unclear what drives folding towards both the monomeric soluble state (a requirement that is essential to protect the PFT producing cell), and the final functional form, which is a heptameric transmembrane spanning pore on the attacked cell. We have investigated folding and the assembly of aerolysin, produced by the human pathogen Aeromonas hydrophila and more specifically the role of the C-terminal propeptide (CTP) and the pore-forming loop for the folding of the soluble and functional pore state. By combining the predictive power of computational techniques (e.g. molecular dynamics simulations) with experimental validation using both structural and functional approaches, we show that the CTP is essential for folding. Aerolysin CTP is crucial in the control of toxic activity since it catalyzes folding of the individual subunits within the bacterium and later controls assembly of the quaternary pore-forming complex at the surface of the target host cell. Based on these data and on the complete characterization of aerolysin domains flexibility we were able to obtain the intermediate pore structure using a newly developed method able to reconstruct the assembly from the structure of the single subunit and low-resolution cryo-EM maps of the final oligomer. These results validated by site directed mutagenesis allowed us to build an accurate structural model of the final functional pore of aerolysin.

2101-Pos Board B87
Viscoelasticity of Hemoglobin S Gels: Moduli, Kinetics, Structure and Mechanisms
Robin W. Briehl, Suzanna Kwong.
Red cell rigidification and damage due to polymerization and gelation of deoxygenated sickle cell hemoglobin lie at the root of pathogenesis in sickle cell disease. Although the abnormal blood rheology and gel viscosity have been studied, viscoelasticity has been little examined. Using sensitive coneplate dynamic rheometry at low shear, we followed development of gels kinetically over 5 decades of increasing elasticity, G' and loss modulus, G", until final levels were reached. Viscoelasticity progresses through 3 regimes. (1) A rapid, brief increase in G' and G" that occurs after initial nucleation can be explained as an initial quadratic dependence on time predicted to result from linear progress of both initiating nucleation and fiber growth with time. The percolation transition from fluid to solid viscoelasticity may also contribute to early rheological change. (2) Following stage (1), G' and G" increase exponentially, consistent with the dominance of heterogeneous nucleation of new fibers on the existing fiber mass. Exponential rates, B=dlnG/dt, scale as the approximate 100th power of solution hemoglobin concentration. The increasing viscoelasticity depends on polymer density, known to increase exponentially, but is also affected by two patterns of interfiber cross-linking and by domain size, packing, overlap and interdigitation. (3) Viscoelasticity reaches asymptotic levels. Final levels of G' and G" have a low, between linear and quadratic, power dependence on gel density. At 15 mM(heme), about 3/4 the normal red cell concentration, G'.150 KPa. This three stage sequence shows more complexity for concentrations near 14 mM heme, with changes in exponential rate during stage (2): slow exponential progress is followed by reacceleration. The slow, near-plateau, regime may depend on entanglements in a rubber-like polymeric system, but with the addition of continuing polymerization that causes residual increase in the plateau modulus, G_e.

2102-Pos Board B88
Minimalistic Approach to Protein Assembly Modelling/Application to the Sickle Cell Hemoglobin Polymerization
Bogdan Barz, Christopher Kepics, Frank A. Ferrone, Brigita Urbanc.
Aberrant assembly of proteins into oligomers and fibrils is associated with many diseases such as Alzheimer's disease, Parkinson's disease, Huntington disease, amyotrophic lateral sclerosis, type II diabetes, systemic amyloidosis, sickle cell anemia, etc. Due to limitations of fully atomistic computational approaches, coarse-grained models provided important insights into protein assembly in general as well as in particular as in the case of amyloid beta-protein, for which discrete molecular dynamics (DMD) using a four-bead protein model with implicit solvent successfully elucidated oligomer formation. Here we addressed the question of how to construct a minimal model of protein assembly using the DMD computational method. We found that a tetrahedron model of a monomer with both effective hydrophobic and hydrophilic interactions accounts for a description of an entire pathway of assembly from monomeric, through transient oligomeric to elongated ordered assemblies.
A more specific and challenging problem is the model for the sickle cell hemoglobin polymerization. A genetic mutation (E6V) on the two beta chains of the hemoglobin tetramer allows effective hydrophobic interactions at these sites with other hydrophobic sites. As a result, the sickle cell hemoglobin polymerizes into long fibers that eventually change the shape of the red blood cell. There are experimentally identified lateral and axial contacts on the mutated hemoglobin that contribute to assembly into a 14-strand fiber structure. Currently there are no computational models to predict the assembly of sickle cell hemoglobin into helical fibers. We have developed a 9-bead DMD model for the sickle cell hemoglobin, based on the crystal-structure sites known to be involved in the assembly. The relative location of the axial and lateral contacts determines the structure of the final fiber as well as intermediate structures observed on the assembly pathway.

2103-Pos Board B89
Nano-Scaled Three-Dimensional Fibrillar Network Made of Curly Amyloid Fibrils of α-Synuclein
Ghibom Bhak, Chul-Seok Hong, Seung R. Paik.
Amyloid fibrils are highly organized protein suprastructures derived from soluble peptides and proteins through the specific self-assembly process. From a single amyloidogenic protein of α-synuclein, two distinctive amyloid fibrils